



## Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact [support@jstor.org](mailto:support@jstor.org).

# THE SPECIFICITY OF STREPTOCOCCI\*

ARTHUR T. HENRICI

*From the Laboratories of the Research Institute of the National Dental Association at the University of Minnesota, Minneapolis*

## INTRODUCTION

The differentiation of streptococci is one of the most perplexing problems of bacteriology. As early as 1884, Rosenbach<sup>1</sup> and Fehleisen<sup>2</sup> claimed for the streptococci which they had isolated, specificity for abscesses and erysipelas respectively, but Petruschky<sup>3</sup> was able to show that the same strain might produce either condition. The more recent work on the specificity of streptococci has developed along several distinct lines.

The carbohydrate-fermentation reactions, which have proved so successful in the differentiation of members of the colon-typhoid group, were first applied to streptococci by Gordon<sup>4</sup> and Houston,<sup>5</sup> whose work was amplified by Andrewes and Horder<sup>6</sup> and in America by Winslow<sup>7</sup> and others.<sup>8</sup> The value of these tests was questioned by Walker,<sup>9</sup> Buerger,<sup>10</sup> Bergey,<sup>11</sup> and Thro,<sup>12</sup> who found the reactions variable; but a general review of the literature indicates that the majority of observers have found the fermentation tests constant enough to warrant a division of the streptococcus group into a number of distinct varieties.

The classification of Schottmüller<sup>13</sup> based on the characters of the colonies on blood-agar plates has been more generally accepted. But even the constancy of the hemolytic property was denied by Ruediger,<sup>14</sup> Anthony,<sup>15</sup> and especially by Rosenow,<sup>16</sup> who found all the members of this group, including the encap-

\* Received for publication May 18, 1916. This work was aided by a grant from the graduate school of the University of Minnesota.

<sup>1</sup> Mikroorganismen bei Wundkrankheiten, 1884.

<sup>2</sup> Aetiologie der Erysipelas, 1883.

<sup>3</sup> Ztschr. f. Hyg. u. Infektionskrankh., 1896, 23, p. 142.

<sup>4</sup> Rep. Local Govt. Board, London, 1903, 33, p. 85. Jour. Path. and Bacteriol., 1911, 15, p. 323.

<sup>5</sup> Rep. Local Govt. Board, London, 1903-4, 33, p. 472; 1904-5, 34, p. 326. Rep. to London County Council on Milk, 1905.

<sup>6</sup> Lancet, 1906, 2, p. 708.

<sup>7</sup> Jour. Infect. Dis., 1912, 10, p. 285.

<sup>8</sup> Winslow and Palmer: Jour. Infect. Dis., 1910, 7, p. 1. Broadhurst: Ibid., 1912, 10, p. 272; 1913, 13, p. 404; 1915, 17, p. 277. Stowell, Hilliard, and Schlesinger: Ibid., 1913, 12, p. 144. Fuller and Armstrong: Ibid., 13, p. 442. Hopkins and Lang: Ibid., 1914, 15, p. 163. Floyd and Wolbach: Jour. Med. Research, 1914, 29, p. 493. Lyall: Ibid., 30, p. 487.

<sup>9</sup> Proc. Roy. Soc. London, 1911, 83, Series B, p. 541.

<sup>10</sup> Jour. Exper. Med., 1907, 9, p. 428.

<sup>11</sup> Jour. Med. Research, 1912, 27, p. 67.

<sup>12</sup> Jour. Infect. Dis., 1914, 15, p. 234; 1915, 17, p. 227.

<sup>13</sup> München. med. Wchnschr., 1903, 1, p. 849; 1910, 57, p. 617.

<sup>14</sup> Jour. Infect. Dis., 1906, 3, p. 663.

<sup>15</sup> Ibid., 1909, 6, p. 332.

<sup>16</sup> Ibid., 1914, 14, p. 1.

sulated forms, readily transmutable. It is to be noted, however, that Holman<sup>17</sup> failed to confirm the results of Rosenow's experiments and pointed out possible sources of error.

Several attempts have been made to throw light on this problem by immunologic studies. Floyd and Wolbach<sup>18</sup> found that agglutination and complement-fixation tests supported the evidence yielded by carbohydrate fermentations. Kligler<sup>19</sup> arrived at similar conclusions from his studies of agglutinins; he found a closer affiliation from an immunologic standpoint between strains fermenting the same carbohydrates than between strains having similar hemolytic properties. Davis,<sup>20</sup> on the other hand, from a study of anaphylaxis in cross-sensitization experiments, found a very close relationship between the hemolytic strains, and in this relationship the nonhemolytic strains did not participate.

Aside from these biochemical studies there has accumulated a considerable amount of literature indicating the occurrence of specific differences between various streptococci in respect to virulence and the localization and characters of the lesions produced. This work had its beginnings in the observations of Poynton and Paine<sup>21</sup> and reached a climax in the recent publications of Rosenow.<sup>22</sup> It is not necessary to review this literature here; references will be made to portions of it in the discussion of my own experiments.

There is very little literature describing experimental work tending to correlate these various series of observations. Andrewes and Horder characterized their *S. pyogenes* and *S. anginosus* as pathogenic and their *S. salivarius* and *S. faecalis* as nonpathogenic. Later Horder<sup>23</sup> noted that *S. salivarius* and *S. faecalis* were frequently associated with malignant endocarditis. Beattie and Yates<sup>24</sup> compared the carbohydrate fermentations with virulence for rabbits; they experienced difficulty in classifying their streptococci, having a large group of variants. Three strains of *S. pyogenes* produced septicemia; 20 strains of *S. salivarius* caused septicemia 6 times, arthritis 10 times, endocarditis once, and no results in 5 rabbits; 6 strains of *S. faecalis* produced septicemia once and arthritis 5 times; 3 strains of *S. anginosus* caused septicemia once and produced no results in 2 rabbits. They concluded that the sugar tests, from the standpoint of sources and pathogenic effects of streptococci, are of no practical value. Floyd and Wolbach<sup>18</sup> noted an inverse relationship between virulence and fermentative powers. Heinemann,<sup>25</sup> also, observed that repeated animal passage, tho raising virulence, decreased the fermentative powers.

Schottmüller found his *S. longus-seu-erysipelatos* associated particularly with phlegmons, erysipelas, and septicemia, and his *S. viridans-seu-mitior* present in localized or subacute infections, especially endocarditis; these findings have been generally confirmed by clinical observations since. Rosenow<sup>26</sup> demonstrated by experimental methods a very close relationship between certain strains of *S. viridans* (which he considers to be mutants of *Pneumococcus*) and endocarditis.

<sup>17</sup> Ibid., 15, p. 293.

<sup>18</sup> Jour. Med. Research, 1914, 29, p. 493.

<sup>19</sup> Jour. Infect. Dis., 1915, 16, p. 327.

<sup>20</sup> Ibid., 1913, 12, p. 386.

<sup>21</sup> Researches on Rheumatism, 1914.

<sup>22</sup> Jour. Am. Med. Assn., 1915, 65, p. 1687.

<sup>23</sup> Quart. Jour. Med., 1908-9, 2, p. 289.

<sup>24</sup> Jour. Path. and Bacteriol., 1911, 16, p. 247.

<sup>25</sup> Jour. Infect. Dis., 1915, 16, p. 221.

<sup>26</sup> Ibid., 1910, 7, p. 411; 1912, 11, p. 210.

Davis<sup>20</sup> published a table indicating that hemolytic streptococci, when inoculated into rabbits, have a pronounced affinity for the joints not shown by the viridans strains, the latter exhibiting a greater affinity for the heart valves.

M'Leod<sup>27</sup> made an exhaustive review of the literature on the relation of hemolysis to virulence in streptococci. Of 16 authors who compared hemolysis with virulence by experimental inoculations into laboratory animals, 4 concluded that a relationship could be established, 10 decided it could not, and 2 drew no conclusions. M'Leod criticized the blood-agar-plate method for determining hemolysis, and stated that if the streptococci to be tested be first grown in the serum of the animal used to determine virulence, and hemolysis be determined by titration with salt suspensions of the red cells of that animal, a close relationship may be established between the hemolytic titer and the minimal lethal dose for that animal. (In my work, hemolysis was determined in every instance on rabbit-blood agar, and rabbits alone were used for inoculation.) Lyall,<sup>28</sup> studying hemolysis by titration with sheep cells, concluded that the hemolytic titer was no absolute criterion of virulence.

It is unfortunate that those bacteriologists who have conducted the greater number of animal experiments with streptococci have paid so little attention to these various biochemical characters. Poynton and Paine, in particular, gave surprisingly meagre descriptions of their *Diplococcus rheumaticus*; but Major<sup>29</sup> identified their organisms with the *S. viridans* of Schottmüller, and both as strains of *S. salivarius* or *S. faecalis* of Andrewes and Horder.

The purpose of the work here reported has been to demonstrate by experimental means what relationship, if any, exists between the various biochemical characters of streptococci (hemolytic power and carbohydrate-fermentation reactions) on the one hand, and virulence, elective organ affinities, and the character of the exudate produced, on the other.

#### MATERIAL AND METHODS

This study was limited to the nonencapsulated forms of streptococci found on the body surfaces or in pathologic conditions in man. These were isolated by plating on agar, and as soon as vigorous pure cultures were obtained, they were planted simultaneously into the various carbohydrate media and into flasks of media to be used for inoculating rabbits. The carbohydrate media, lactose, saccharose, mannite, raffinose, salicin, and inulin, were prepared according to the method of Holman,<sup>30</sup> using Andrade's indicator. Hemolysis was determined by plating on rabbit-blood agar (2 drops to the cubic centimeter), usually from the broth cultures used to inoculate the rabbits, thus being insured the purity of the cultures injected. The time of cultivation of the streptococci on artificial media before their inoculation into rabbits averaged 7 days.

Rabbits were injected with the growth from 24-hour cultures in double-strength 2%-dextrose broth to which had been added one-fourth volume of sterile beef serum or human ascitic fluid. As far as possible, young rabbits weighing close to 1,000 gm. were used, but the size varied considerably. Inoculations were

<sup>27</sup> Jour. Path. and Bacteriol., 1915, 19, p. 392.

<sup>28</sup> Jour. Med. Research, 1914, 30, p. 515.

<sup>29</sup> Bull. Johns Hopkins Hosp., 1912, 23, p. 326.

<sup>30</sup> Jour. Infect. Dis., 1914, 14, p. 209.

made in multiples, usually 4 rabbits being injected with the same strain in varying doses. When doses of more than 10 c.c. were given, the centrifugated bacteria, free from broth, were used. In the earlier part of the work, massive doses (as high as 75 c.c.) were injected, as recommended by Rosenow. It was found, however, that many of the rabbits receiving these large doses died in a very short time, apparently from toxemia; and where less virulent cultures were injected, the rabbits receiving very large doses were not much more likely to develop lesions than those inoculated with moderate doses, so that the massive doses were abandoned. Beattie<sup>31</sup> has found that with rheumatic strains the dose is of little importance as far as the development of arthritis is concerned. It was thought desirable, however, to vary the dose somewhat in order to obtain a rough titer of virulence. The average dose was 5 c.c. All injections were made intravenously.

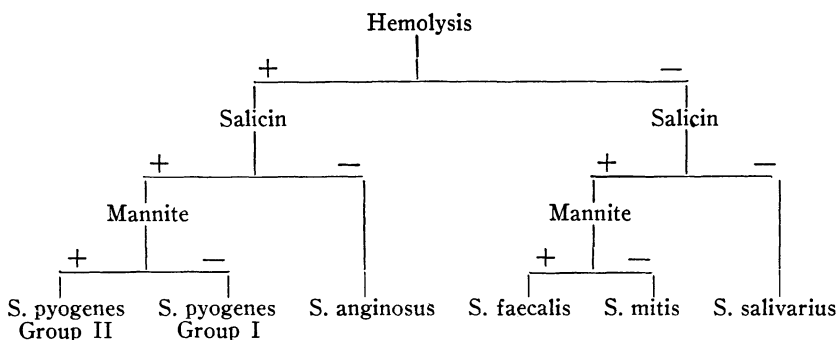
If the animals did not die spontaneously, they were chloroformed at intervals of from 2 to 10 days, as previous experience had shown that many of the lesions produced are more or less evanescent, a majority of the rabbits tending to recover completely from their infections. Except for a few instances, complete autopsies were made. In the case of the first 100 rabbits, routine microscopical examination was made of the arch of the aorta, the heart muscle, spleen, pancreas, liver, gallbladder, appendix, kidneys, and one or more of the lymph nodes, as well as of all other tissues showing gross lesions. I learned from these experiences that lesions were seldom found microscopically which did not appear grossly, so that in subsequent work, I made microscopical examinations only in those rabbits showing some gross evidence of infection, still examining, however, sections of all the organs named. I did not make routine bacteriologic examinations of these rabbits; they were made frequently enough, however, to indicate that the streptococci were present in most of the lesions described. The organisms recovered invariably showed the same fermentative and hemolytic characters as those inoculated.

#### SOURCES AND CLASSIFICATION OF STRAINS

Cultures were derived from many sources. An effort was made to obtain strains of each variety, both those existing as saprophytes on the body surfaces and those exhibiting varying degrees of pathogenicity within the tissues. There is some disproportion in number among the strains of each variety. Thus, as a consequence of the fact that during the course of this work I was also investigating chronic mouth infections, a large number of strains of *S. mitis* from that source are included. I experienced difficulty in finding pathogenic strains of *S. faecalis* and *S. anginosus*. The source of each strain is indicated in tables to follow.

In classifying the strains I adopted the terminology of Andrewes and Horder. A classification based on the fermentation reactions alone presents considerable difficulties; thus, the only criteria offered for the differentiation of *S. mitis* and *S. pyogenes* are the length of the chains and the pathogenicity for mice, both of which are probably highly variable characters. If the sugar tests be considered in relation to the presence or absence of hemolysis, however, the classification becomes relatively simple. The terminology used is indicated in the following diagram:

<sup>31</sup> Jour. Path. and Bacteriol., 1910, 14, p. 432.



As will be seen, it was found desirable to divide *S. pyogenes* into 2 groups. Group II differs from the type species in fermenting mannite. By the sugar tests alone these would be classified as *S. faecalis*, and Lyall<sup>28</sup> suggested that these strains represent varieties of *S. faecalis* which have acquired hemolytic properties,, but there seems to be no clear evidence for this. Practically all strains fermented lactose and saccharose and acidified litmus milk. Only 2 strains fermented inulin; they failed to produce capsules in mice and were classified as variants of *S. salivarius* and *S. mitis* respectively. Raffinose, according to Andrewes and Horder's tables, is fermented regularly only by *Pneumococcus*. In our series it was usually fermented by the strains classified as *S. salivarius*, and by most strains of *S. mitis*, but seemed to serve no useful purpose in the classification and was therefore not considered.

#### TABULATION OF RESULTS

In Tables 1 to 6 the essential details of the various experiments are presented in condensed form. In the column marked "Died or Chloroformed" the sign + indicates that the animals died spontaneously within the time limits of the experiments. In the columns indicating the distribution of the various lesions, the sign + indicates an infiltrative lesion, and H indicates an interstitial hemorrhage.

#### DESCRIPTION AND DISCUSSION OF LESIONS

The lesions produced may be divided into 2 groups, hemorrhagic and infiltrative. The hemorrhagic lesions (the importance of which has been emphasized by Rosenow) occurred in the capsular tissues of the joints, in the voluntary muscles, heart valves, and occasionally in the heart muscle and in the kidneys. In several instances hemorrhages also were noted in the stomach, in the small intestine, and in the appendix. For the most part these hemorrhages were small, but occasionally they were extensive, involving for instance all the muscles of one extremity. Microscopically, these lesions usually showed nothing but interstitial hemorrhages; occasionally, however, the hemorrhagic

areas contained small foci of leukocytic infiltration, so that it seems reasonable to suppose that the hemorrhagic condition precedes the infiltrative lesion. No attention was paid to parenchymatous changes, such as fatty infiltration in the heart, cloudy swelling of the kidneys, etc.

*Nervous System.*—The central nervous system was not examined routinely in these rabbits, but some of them presented certain nervous symptoms and lesions in the brain which are interesting when considered in connection with the published reports on the relation of streptococci to chorea.

Dana<sup>32</sup> observed diplococci in sections of the brain in chorea, and Apert<sup>33</sup> cultivated a diplococcus from the blood. Wassermann, Westphal, and Malkoff<sup>34</sup> isolated a diplostreptococcus from the blood and from the brain in a fatal case of chorea; this organism produced arthritis in rabbits. Poynton and Paine<sup>31</sup> repeatedly cultivated their "diplococcus" from the blood and spinal fluid in cases of chorea and were able to demonstrate the organism in the pia matter and in the vessel walls of the brain. They also noted the occurrence of choreic symptoms in one of a series of rabbits inoculated with streptococci from rheumatism, and found the organism in the rabbit's brain. Beattie,<sup>35</sup> too, observed choreic movements in an inoculated rabbit, and found an exudate of polymorphonuclear cells on the surface of the cord in places. Cole<sup>36</sup> noted the occurrence of convulsive incoordinate movements in 2 rabbits injected with streptococci from cases of septicemia, but did not believe that the symptoms resembled those of chorea.

The pathologic changes occurring in rheumatic chorea are not well known. Poynton and Holmes<sup>37</sup> described vascular changes, hyperemia, multiple thromboses with softening, and, more constantly, perivascular small-round-cell infiltration in the pia and in the brain substance; also degenerative changes in the nerve cells and fibers.

The symptoms shown by our rabbits, which were probably identical with those described by the investigators quoted, may be best characterized as an inability to keep still. There were constant movements of the head from side to side, and in walking there was marked staggering, with a very apparent inability to coordinate movements. Retraction of the neck was noted in one animal.

Grossly the brains appeared normal, but microscopically there were found in all 4 rabbits lesions which varied only in degree. In the pia there were congestion and occasional microscopic hemorrhages, with a cellular infiltration that varied from a few perivascular lymphocytes in one case to a diffuse infiltration of the entire brain surface in others. This layer of inflammatory cells was thin, and altho it showed numerous pus cells in many places, mononuclear cells predominated. Within the brain substance there were areas of peri-

<sup>32</sup> Am. Jour. Med. Sc., 1894, 108, p. 31.

<sup>33</sup> Compt. rend. Soc. de biol., 1898, 5, p. 128.

<sup>34</sup> Berl. klin. Wchnschr., 1899, 36, p. 638.

<sup>35</sup> Jour. Path. and Bacteriol., 1904, 9, p. 272.

<sup>36</sup> Jour. Infect. Dis., 1904, 1, p. 714.

<sup>37</sup> Lancet, 1906, 2, p. 982.

TABLE 1  
STREPTOCOCCUS PYOGENES, GROUP I

| Strain | Source                                       | Days Cultivated | Rabbit  | Dose in c.c.                      | Duration of Infection, Days     | Died or Chloroformed*           |
|--------|--|-----------------|---|-----------------------------------|---------------------------------|---------------------------------|
| 6      | Nose.....                                    | 4               | 30<br>31<br>32                                | 5<br>15<br>45                     | 5<br>5<br>5                     | 0<br>0<br>0                     |
| 33     | Sputum.....                                  | 6               | 107<br>108<br>109<br>110                      | 45<br>15<br>5<br>5                | 1<br>1<br>2<br>2                | +<br>+<br>+<br>+                |
| 44     | Dental fistula.....                          | 5               | 152<br>153<br>154<br>155                      | 10<br>5<br>5<br>5                 | 0<br>5<br>5<br>5                | +<br>0<br>+<br>0                |
| 48     | Tonsil.....                                  | 17              | 156<br>157<br>159<br>166                      | 6<br>2<br>8<br>4                  | 1<br>1<br>1<br>2                | +<br>+<br>+<br>+                |
| 65     | Blood culture (septicemia of obscure origin) | 7               | 168<br>169<br>170                             | 10<br>5<br>8                      | 8<br>8<br>8                     | 0<br>0<br>0                     |
| 96     | Empyema.....                                 | 1               | 205<br>206<br>207<br>208<br>209<br>210<br>211 | 5<br>5<br>5<br>5<br>1<br>1<br>1   | 1<br>1<br>1<br>1<br>3<br>3<br>3 | +<br>+<br>+<br>+<br>+<br>+<br>+ |
| 110    | Blood culture (septicemia).....              | 4               | 217<br>218<br>219<br>220<br>221               | 5<br>3<br>1<br>1<br>$\frac{1}{2}$ | 4<br>4<br>4<br>4<br>4           | 0<br>0<br>0<br>0<br>0           |
| 112    | Blood culture (septicemia).....              | 1               | 226<br>227<br>228<br>229                      | 5<br>5<br>3<br>1                  | 5<br>5<br>4<br>5                | 0<br>0<br>0<br>0                |
| 114    | Knee-joint (arthritis following pneumonia)   | 1               | 234<br>235<br>236<br>237                      | 8<br>5<br>5<br>1                  | 1<br>6<br>8<br>8                | +<br>+<br>0<br>0                |
| 122    | Peritonitis.....                             | 1               | 238<br>239<br>240<br>241                      | 10<br>5<br>3<br>1                 | 1<br>2<br>2<br>7                | +<br>+<br>+<br>0                |
| 123    | Acute endocarditis following erysipelas      | 1               | 242<br>243<br>244<br>245                      | 10<br>5<br>5<br>1                 | 4<br>6<br>6<br>6                | +<br>0<br>0<br>0                |

\* In this column the sign + indicates that the animal died spontaneously within the time limits of the experiment. In the columns showing the distribution of the various lesions, the sign + indicates an infiltrative lesion, and H indicates an interstitial hemorrhage. This key applies to all of Tables 1 to 6.



TABLE 1—Continued  
STREPTOCOCCUS PYOGENES, GROUP I

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks                        |
|--------------|--------------|--------|-------------------|-------------|--------|---------|--------------------------------|
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | +           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | +           | 0      | 0       | Subacute meningitis ("chorea") |
| 0            | 0            | 0      | 0                 | +           | +      | +       |                                |
| 0            | 0            | H      | 0                 | 0           | 0      | +       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Died in a few hours            |
| +            | +            | +      | 0                 | 0           | 0      | +       |                                |
| +            | +            | 0      | 0                 | 0           | 0      | +       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | +      | 0       |                                |
| +            | 0            | 0      | 0                 | 0           | +      | 0       |                                |
| 0            | H            | 0      | 0                 | 0           | +      | 0       | Hemorrhages in appendix        |
| 0            | 0            | 0      | 0                 | 0           | +      | 0       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| +            | 0            | +      | +                 | 0           | 0      | 0       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | +      | +                 | 0           | 0      | +       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | +       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Fibrinous pericarditis         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | +      | 0                 | 0           | 0      | +       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| +            | +            | 0      | 0                 | +           | +      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Sero-fibrinous pericarditis    |
| +            | +            | 0      | 0                 | 0           | 0      | 0       |                                |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |                                |
| 0            | 0            | 0      | 0                 | 0           | 0      | +       |                                |

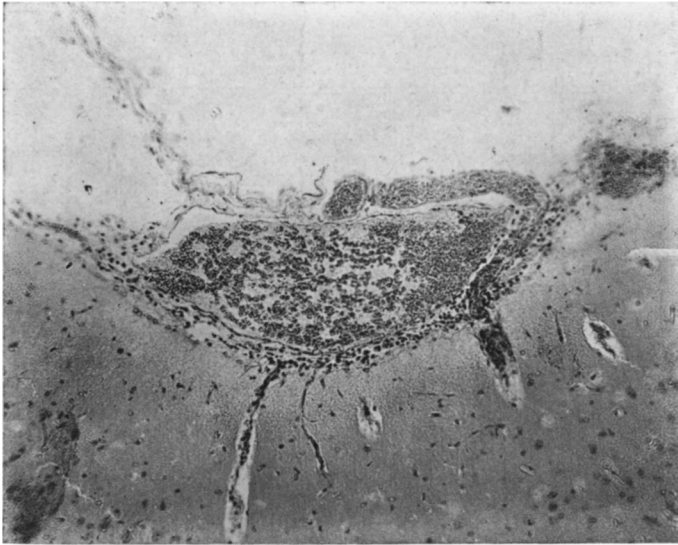


Fig. 1. "Chorea." Perivascular round-cell infiltration in the pia mater.

vascular lymphocytic infiltration, and minute foci of mononuclear cells not associated with vessels. Beneath the ependyma of the ventricles there was a diffuse lymphocytic infiltration. These changes were not confined to the cerebrum but were present in the cerebellum and pons as well. The condition may be considered a subacute encephalomeningitis.

It is not certain that either the symptoms or the lesions in these rabbits have anything in common with rheumatic chorea in man. In any event, the condition cannot be considered specific, since it was produced once by *S. pyogenes*, once by *S. mitis*, and twice by *S. salivarius*.

*Heart Valves.*—The infectious nature of endocarditis has long been known, and a variety of organisms have been associated with this disease; of these, streptococci have been by far the most frequent.

The more or less specific characters of these streptococci were established by the studies of Schottmüller,<sup>18</sup> Horder,<sup>20</sup> and Libman and Cellar.<sup>21</sup> Poynton and Paine<sup>21</sup> also cultivated streptococci from cases of endocarditis which they considered identical with those obtained from rheumatism; further, they obtained the same organism from cases of malignant endocarditis and were able to produce this type of disease in rabbits with streptococci from cases of simple rheumatism. They concluded that their work had established firmly the relationship between rheumatism and both simple and malignant endo-

<sup>28</sup> Am. Jour. Med. Sc., 1910, 140, p. 516.

carditis. In this connection it is interesting to note that Thalheimer and Rothschild<sup>39</sup> failed to find the specific lesions of rheumatic myocarditis in cases of endocarditis due to *S. viridans*.

Endocarditis has been produced experimentally in animals by a number of observers. Wyssokowitsch<sup>40</sup> found that intravenous injections of staphylococci and streptococci into rabbits failed to produce endocarditis unless the valves had previously been injured by operative manipulation (this manipulation, as had been shown by Rosenbach,<sup>41</sup> did not in itself produce inflammatory changes in the valves of rabbits) and Orth<sup>42</sup> interpreted these experiments as indicating that the disease required for its development not only the presence of circulating micro-organisms, but also a predisposing factor in the valves themselves. The experiments of Wyssokowitsch were repeated and confirmed by Fraenkel and Sänger<sup>43</sup> and by Weichselbaum.<sup>44</sup> Prudden<sup>45</sup> accomplished the same end by previously treating the valves with caustics. Ribbert<sup>46</sup> produced endocarditis by injecting staphylococci simultaneously with an emulsion of potato, again producing a predisposing condition, according to Orth's interpretation, either in the adherence of the potato particles to the valves and chordae or in the production of emboli. Bonome,<sup>47</sup> however, observed the development of endocarditis in rabbits merely injected with cultures of staphylococci, if these formed clumps large enough to constitute emboli. Successful results with staphylococci were also obtained by Jossierand and Roux<sup>48</sup> and by Lissauer.<sup>49</sup>

Dreschfeld,<sup>50</sup> apparently, was the first to succeed in producing endocarditis by the simple inoculation of streptococci into rabbits. More recently, such observations have been repeatedly made by Poynton and Paine, Beattie,<sup>51</sup> Shaw,<sup>51</sup> and Libman and Cellar.<sup>52</sup> Rosenow has been especially successful with these experiments. Thus, 84% of his rabbits inoculated with streptococci from endocarditis have shown that lesion at autopsy, as compared with 14% of rabbits inoculated with strains from other sources.<sup>52</sup> The apparent affinity of these streptococci (which he considers to be modified pneumococci) for the heart valves, Rosenow believes is related to their ability to produce clumps in liquid, and adherent colonies on solid media; for he finds that endocarditis in rabbits is primarily an embolic process, the organisms lodging within capillaries in the valves and producing first a hemorrhage, later inflammatory infiltration, ending in ulceration and the development of vegetations.<sup>52</sup>

In discussing the heart-valve lesions in our rabbits, then, two questions present themselves for consideration: (1) To what extent do streptococci of various kinds exhibit an affinity for the heart valves,

<sup>39</sup> Jour. Exper. Med., 1914, 19, p. 417.

<sup>40</sup> Virchow's Arch. f. path. Anat., 1886, 103, p. 301.

<sup>41</sup> Arch. f. exper. Path. u. Pharmacol., 1878, 9, p. 1.

<sup>42</sup> Virchow's Arch. f. path. Anat., 1886, 103, p. 333.

<sup>43</sup> Ibid., 1887, 108, p. 286.

<sup>44</sup> Centralbl. f. Bakteriöl., 1887, 2, p. 209.

<sup>45</sup> Am. Jour. Med. Sc., 1887, 93, p. 55.

<sup>46</sup> Fortschr. d. Med., 1886, 4, p. 1.

<sup>47</sup> Arch. ital. de biol., 1887, 8, p. 10.

<sup>48</sup> Arch. de méd. exper. et d'anat. path., 1892, 4, p. 469.

<sup>49</sup> Centralbl. f. allg. Path. u. path. Anat., 1912, 23, p. 243.

<sup>50</sup> Brit. Med. Jour., 1887, 2, p. 887.

<sup>51</sup> Jour. Path. and Bacteriol., 1904, 9, p. 158.

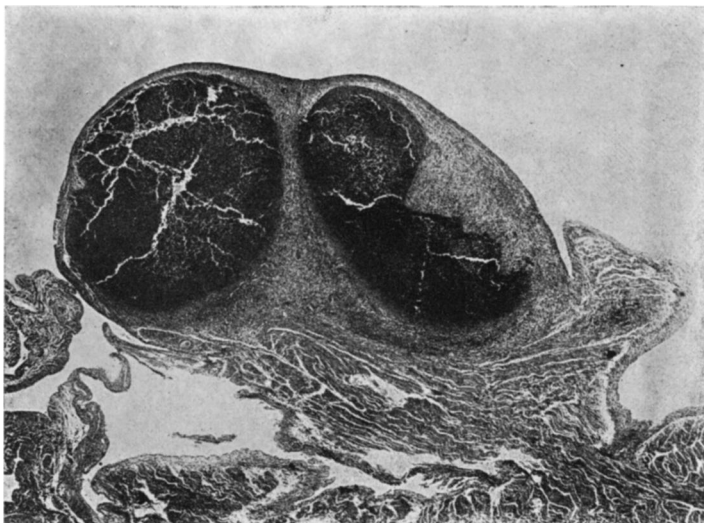


Fig. 2. Embolic endocarditis. Abscesses have developed within the valve leaflet, surrounded by an intact endothelial surface.

and (2) does the process begin primarily as an embolism within the valve, or as an implantation on the endothelial surface itself?

The valve lesions obtained were either subendothelial hemorrhages (which would not disappear after vigorous washing) or vegetations. Microscopical examination was not made of all the vegetations, several of the hearts being saved as gross specimens. In one case, at least, what appeared grossly as vegetations proved microscopically to be abscesses within the valve, which had developed from the base of the valve, spreading towards its free border and pushing the surfaces apart, so that they were completely surrounded by an intact endothelial surface; here undoubtedly the process developed by embolism but did not begin primarily in the valve itself. In all the other instances, however, there was found on the surface of the valve an exudate of fibrin with leukocytes and masses of cocci, the valve itself showing but little change, certainly no evidences that the process had begun within and later ulcerated through (Fig. 3). We must conclude then that streptococcal endocarditis usually develops by implantation on the surface of the valve.

Our experiments have not revealed specific differences in the affinities of the various classes of streptococci for the heart valves. *S. mitis* produced hemorrhages 4 times and vegetations 3 times; *S. salivarius*, hemorrhage once and vegetations 4 times; *S. faecalis*, vegeta-



Fig. 3. Endocarditis. Vegetation attached to the tip of the valve cusp, the valve structure itself being intact.

tions once. *S. pyogenes* of Group I, caused hemorrhages twice and vegetations 3 times; of Group II, hemorrhage once and vegetations 3 times; *S. anginosus*, vegetations once. Lesions of the heart valves, then, developed in about 9% of the rabbits inoculated with nonhemolytic strains, and in 11% of those which received hemolytic streptococci.

*Heart Muscle.*—Interest in the myocardial lesions produced by streptococci depends on the fact that there occurs in rheumatic fever a type of myocarditis which is apparently specific for that disease.

This lesion has been described by Aschoff,<sup>52</sup> Geipel,<sup>53</sup> Fraenkel,<sup>54</sup> Coombs,<sup>55</sup> Thalheimer and Rothschild,<sup>56</sup> and others. The reaction occurs in circumscribed areas, called submiliary nodules or Aschoff-Geipel bodies. They are found in the intermuscular septa, especially about the vessels. They are composed of large oval or spindle-shaped cells arranged about the vessels in rosettes, or between the muscle fibers in fusiform areas. The nodules are more frequent, according to Coombs, near the endocardium, especially at the bases of the valves. The cells forming these nodules, as shown by Fraenkel, stain red with pyronin-methyl green. Multinucleated giant cells also occur. Coombs found similar lesions in the articular tissues and subcutaneous nodules in cases of rheumatism.

<sup>52</sup> Verhandl. d. deutsch. path. Gesellsch., 1904, 8, p. 46.

<sup>53</sup> Deutsch. Arch. f. klin. Med., 1905, 85, p. 75.

<sup>54</sup> Ziegler's Beiträge, 1912, 52, p. 597.

<sup>55</sup> Jour. path. and Bacteriol., 1910, 15, p. 489. Quart. Jour. Med., 1908-9, 2, p. 26.

TABLE 2  
STREPTOCOCCUS PYOGENES, GROUP II

| Strain | Source                                     | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days | Died or Chloroformed* |
|--------|--|-----------------|--------|--------------|-----------------------------|-----------------------|
| 32     | Sputum (pulmonary abscess)....             | 6               | 103    | 45           | 5                           | +                     |
|        |  |                 | 104    | 3            | 5                           | 0                     |
|        |  |                 | 105    | 5            | 5                           | 0                     |
|        |  |                 | 106    | 5            | 5                           | 0                     |
| 45     | Ascites fluid.....                         | 5               | 144    | 10           | 0                           | +                     |
|        |  |                 | 145    | 5            | 4                           | +                     |
|        |  |                 | 146    | 5            | 5                           | 0                     |
|        |  |                 | 147    | 5            | 3                           | +                     |
| 38     | Empyema.....                               | 6               | 148    | 10           | 3                           | +                     |
|        |  |                 | 149    | 5            | 3                           | +                     |
|        |  |                 | 150    | 5            | 5                           | +                     |
|        |  |                 | 151    | 5            | 3                           | +                     |
| 111    | Empyema (traumatic).....                   | 2               | 222    | 5            | 4                           | 0                     |
|        |  |                 | 223    | 3            | 5                           | 0                     |
|        |  |                 | 224    | 1            | 4                           | 0                     |
|        |  |                 | 225    | 1            | 5                           | 0                     |
| 113    | Meningitis (secondary to decubitus ulcers) | 1               | 230    | 3            | 5                           | 0                     |
|        |  |                 | 231    | 5            | 4                           | 0                     |
|        |  |                 | 232    | 5            | 4                           | 0                     |
|        |  |                 | 233    | 1            | 5                           | 0                     |

\* See Table 1 for the significance of + and H.

TABLE 3  
STREPTOCOCCUS ANGINOSUS

| Strain | Source                                       | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days | Died or Chloroformed* |
|--------|--|-----------------|--------|--------------|-----------------------------|-----------------------|
| 109    | Blood culture (septicemia of obscure origin) | 4               | 212    | 3            | 4                           | 0                     |
|        |  |                 | 213    | 1            | 4                           | 0                     |
|        |  |                 | 214    | 5            | 4                           | 0                     |
|        |  |                 | 215    | 1            | 4                           | 0                     |
|        |  |                 | 216    | 1            | 4                           | 0                     |
| 124    | Throat (scarlet fever).....                  | 3               | 246    | 10           | 2                           | +                     |
|        |  |                 | 247    | 5            | 2                           | +                     |
|        |  |                 | 248    | 5            | 6                           | 0                     |
|        |  |                 | 249    | 1            | 2                           | +                     |
| 125    | Throat (scarlet fever).....                  | 3               | 250    | 10           | 5                           | +                     |
|        |  |                 | 251    | 5            | 2                           | +                     |
|        |  |                 | 252    | 5            | 1                           | +                     |
|        |  |                 | 253    | 1            | 2                           | +                     |
| 126    | Throat (scarlet fever).....                  | 3               | 254    | 10           | 1                           | +                     |
|        |  |                 | 255    | 5            | 4                           | 0                     |
|        |  |                 | 256    | 5            | 1                           | +                     |
|        |  |                 | 257    | 1            | 4                           | 0                     |
| 127    | Throat (scarlet fever).....                  | 3               | 258    | 10           | 4                           | 0                     |
|        |  |                 | 259    | 5            | 4                           | 0                     |
|        |  |                 | 260    | 5            | 4                           | 0                     |
|        |  |                 | 261    | 1            | 4                           | 0                     |
| 128    | Throat (simple angina).....                  | 2               | 262    | 10           | 1                           | +                     |
|        |  |                 | 263    | 5            | 3                           | 0                     |
|        |  |                 | 264    | 5            | 1                           | +                     |
|        |  |                 | 265    | 1            | 3                           | 0                     |
|        |  |                 | 266    | 10           | 2                           | 0                     |

\* See Table 1 for the significance of + and H.

STREPTOCOCCUS PYOGENES, GROUP II

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks   |
|--------------|--------------|--------|-------------------|-------------|--------|---------|---|
| 0            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Died in an hour   |
| +            | 0            | +      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| +            | +            | 0      | 0                 | 0           | +      | +       | Multiple septic infarcts of spleen<br>Acute peritonitis |
| +            | 0            | 0      | 0                 | 0           | +      | +       |   |
| +            | +            | 0      | 0                 | 0           | +      | +       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| +            | H            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | +                 | 0           | 0      | +       |   |
| +            | 0            | 0      | +                 | 0           | 0      | +       |   |
| 0            | +            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |

TABLE 3—Continued

STREPTOCOCCUS ANGINOSUS

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks                          |
|--------------|--------------|--------|-------------------|-------------|--------|---------|----------------------------------|
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | +                 | 0           | 0      | 0       |                                  |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | ?            | 0      | 0                 | 0           | 0      | 0       |                                  |
| +            | 0            | 0      | 0                 | +           | +      | 0       | Thymus congested and hemorrhagic |
| +            | 0            | +      | +                 | +           | 0      | 0       | Thymus congested and hemorrhagic |
| 0            | 0            | 0      | 0                 | +           | +      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | ?            | 0      | +                 | 0           | 0      | +       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | +      | +                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | +            | 0      | 0                 | 0           | 0      | +       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| +            | 0            | +      | +                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |                                  |
| +            | 0            | +      | 0                 | 0           | 0      | +       | Hemorrhage in stomach            |

TABLE 4  
STREPTOCOCCUS MITIS

| Strain | Source                                | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days | Died or Chloroformed* |
|--------|---------------------------------------|-----------------|--------|--------------|-----------------------------|-----------------------|
| 1      | Tonsil.....                           | 21              | 1      | 1            | 2                           | 0                     |
|        |                                       |                 | 2      | 5            | 2                           | 0                     |
|        |                                       |                 | 3      | 5            | 2                           | 0                     |
|        |                                       |                 | 4      | 5            | 3                           | 0                     |
|        |                                       |                 | 5      | 5            | 4                           | 0                     |
|        |                                       |                 | 6      | 5            | 6                           | 0                     |
|        |                                       |                 | 7      | 5            | 7                           | 0                     |
|        |                                       |                 | 8      | 15           | 2                           | 0                     |
|        |                                       |                 | 9      | 45           | 2                           | 0                     |
| 2      | Saliva.....                           | 3               | 14     | 1            | 2                           | 0                     |
|        |                                       |                 | 15     | 5            | 2                           | 0                     |
|        |                                       |                 | 16     | 45           | 2                           | 0                     |
|        |                                       |                 | 17     | 15           | 2                           | 0                     |
| 4      | Blood culture (subacute endocarditis) | 2               | 22     | 1            | 2                           | 0                     |
|        |                                       |                 | 23     | 5            | 2                           | 0                     |
|        |                                       |                 | 24     | 15           | 2                           | 0                     |
|        |                                       |                 | 25     | 45           | 2                           | 0                     |
| 7      | Saliva.....                           | 8               | 33     | 1            | 4                           | 0                     |
|        |                                       |                 | 34     | 5            | 2                           | +                     |
|        |                                       |                 | 35     | 15           | 4                           | 0                     |
|        |                                       |                 | 36     | 45           | 0                           | +                     |
| 8      | Saliva.....                           | 8               | 37     | 3            | 6                           | 0                     |
|        |                                       |                 | 38     | 5            | 6                           | 0                     |
|        |                                       |                 | 39     | 15           | 6                           | 0                     |
|        |                                       |                 | 40     | 45           | 0                           | 0                     |
| 13     | Dental abscess.....                   | 21              | 65     | 5            | 6                           | 0                     |
|        |                                       |                 | 66     | 5            | 6                           | 0                     |
|        |                                       |                 | 67     | 5            | 6                           | 0                     |
|        |                                       |                 | 68     | 5            | 6                           | 0                     |
| 16     | Dental abscess.....                   | 4               | 61     | 5            | 4                           | 0                     |
|        |                                       |                 | 62     | 5            | 1                           | +                     |
|        |                                       |                 | 63     | 5            | 4                           | 0                     |
|        |                                       |                 | 64     | 5            | 10                          | +                     |
| 20     | Dental abscess .....                  | 4               | 57     | 5            | 4                           | 0                     |
|        |                                       |                 | 58     | 5            | 4                           | 0                     |
|        |                                       |                 | 59     | 5            | 4                           | 0                     |
|        |                                       |                 | 60     | 5            | 4                           | 0                     |
| 29     | Dental abscess.....                   | 7               | 91     | 5            | 4                           | 0                     |
|        |                                       |                 | 92     | 10           | 4                           | 0                     |
|        |                                       |                 | 93     | 8            | 4                           | 0                     |
|        |                                       |                 | 94     | 8            | 4                           | 0                     |
| 30     | Dental abscess.....                   | 7               | 95     | 10           | 4                           | 0                     |
|        |                                       |                 | 96     | 5            | 3                           | +                     |
|        |                                       |                 | 97     | 7            | 4                           | 0                     |
|        |                                       |                 | 98     | 5            | 4                           | 0                     |
| 31     | Dental abscess.....                   | 6               | 99     | 5            | 4                           | 0                     |
|        |                                       |                 | 100    | 10           | 4                           | 0                     |
|        |                                       |                 | 101    | 8            | 4                           | 0                     |
|        |                                       |                 | 102    | 8            | 4                           | 0                     |
| 34     | Sputum.....                           | 6               | 111    | 45           | 5                           | 0                     |
|        |                                       |                 | 112    | 5            | 3                           | +                     |
|        |                                       |                 | 113    | 5            | 5                           | 0                     |
|        |                                       |                 | 114    | 5            | 5                           | 0                     |



## STREPTOCOCCUS MITIS

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks   |
|--------------|--------------|--------|-------------------|-------------|--------|---------|---|
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | H                 | 0           | 0      | 0       |   |
| +            | H            | 0      | 0                 | +           | +      | 0       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | +            | 0      | +                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| H            | 0            | 0      | 0                 | +           | +      | 0       | Lesions in adventitia of aorta                                |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       | Hemorrhages in small intestine                                |
| +            | 0            | 0      | +                 | 0           | 0      | +       | Hemorrhages in stomach  |
| +            | 0            | 0      | 0                 | 0           | 0      | +       | Lesions in aorta; millary abscesses in lungs                  |
| 0            | 0            | +      | 0                 | 0           | +      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | H      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Died in 10 minutes  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | +      | 0                 | 0           | 0      | +       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | 0      | 0                 | +           | +      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       | Fibrinous peritonitis, organizing                             |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | H                 | 0           | 0      | +       |   |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | H                 | 0           | 0      | +       | Focal encephalitis ("chorea"). Lesions in adventitia of aorta |
| +            | 0            | 0      | +                 | 0           | 0      | +       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | H            | +      | H                 | 0           | 0      | +       |   |
| +            | 0            | +      | H                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | +            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | 0      | 0                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |   |
| +            | 0            | 0      | H                 | 0           | 0      | +       |   |
| 0            | 0            | 0      | +                 | 0           | 0      | 0       | Lesions in media of aorta                                     |

TABLE 4—*Continued*  
STREPTOCOCCUS MITIS

| Strain | Source                                    | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days | Died or Chloroformed* |
|--------|---|-----------------|--------|--------------|-----------------------------|-----------------------|
| 39     | Feces.....                                | 3               | 124    | 30           | 7                           | 0                     |
|        |   |                 | 125    | 5            | 7                           | 0                     |
|        |   |                 | 126    | 10           | 7                           | 0                     |
|        |   |                 | 127    | 4            | 7                           | 0                     |
| 41     | Feces.....                                | 3               | 132    | 30           | 7                           | 0                     |
|        |   |                 | 133    | 10           | 7                           | 0                     |
|        |   |                 | 134    | 8            | 7                           | 0                     |
|        |   |                 | 135    | 5            | 7                           | 0                     |
| 43     | Feces.....                                | 3               | 140    | 30           | 7                           | 0                     |
|        |   |                 | 141    | 10           | 7                           | 0                     |
|        |   |                 | 142    | 8            | 7                           | 0                     |
|        |   |                 | 143    | 5            | 7                           | 0                     |
| 63     | Blood culture (malignant endocarditis)    | 7               | 161    | 6            | 2                           | +                     |
|        |   |                 | 162    | 5            | 3                           | 0                     |
|        |   |                 | 158    | 7            | 1                           | +                     |
|        |   |                 | 167    | 5            | 2                           | +                     |
| 93     | Ankle joint (chronic deforming arthritis) | 3               | 185    | 5            | 2                           | +                     |
|        |   |                 | 186    | 5            | 2                           | +                     |
|        |   |                 | 187    | 5            | 2                           | +                     |
|        |   |                 | 188    | 5            | 7                           | 0                     |
|        |   |                 | 189    | 5            | 7                           | 0                     |

\* See Table 1 for the significance of + and H.

Since this is the only lesion occurring in rheumatic fever which appears to be absolutely specific, it is important to learn whether streptococci of the type cultivated from cases of rheumatism by Poynton and Paine will produce such lesions in rabbits, and especially whether the ability to produce these lesions is peculiar to this type of streptococci.

Bracht and Wächter<sup>56</sup> inoculated streptococci from two cases of rheumatism in which Aschoff-Geipel bodies were found, into a small series of rabbits. Some of the animals developed areas of focal myocarditis composed mainly of lymphocytes; and, altho the Aschoff type of lesion did not occur, they considered it significant that the lesions were of this more or less chronic type rather than in the form of the miliary abscesses which they obtained with *S. pyogenes*. Jackson<sup>57</sup> described myocardial lesions occurring in rabbits inoculated with streptococci from the Chicago milk-borne epidemic of sore throat, and with *S. viridans* from cases of endocarditis. She noted the presence of lesions composed of large mononuclear cells, and in the oldest lesions giant cells, and she considered the condition to be markedly similar to rheumatic myocarditis. Staining with pyronin-methyl green is not mentioned. It is to be noted that the sore-throat streptococci were hemolytic, and that altho they presented cer-

<sup>56</sup> Deutsch. Arch. f. klin. Med., 1909, 96, p. 493.<sup>57</sup> Jour. Infect. Dis., 1912, 11, p. 243.

## STREPTOCOCCUS MITIS

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks |
|--------------|--------------|--------|-------------------|-------------|--------|---------|---------|
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |         |
| +            | 0            | +      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | H                 | 0           | +      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |
| +            | H            | 0      | 0                 | +           | 0      | +       |         |
| 0            | 0            | 0      | H                 | +           | 0      | 0       |         |
| +            | H            | 0      | 0                 | 0           | +      | +       |         |
| 0            | +            | 0      | 0                 | 0           | 0      | 0       |         |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |         |

tain peculiarities, they could not be differentiated by sugar tests from *S. pyogenes*. (The carbohydrate-fermentation reactions of milk-epidemic sore-throat streptococci were studied by Smith and Brown.<sup>88</sup>) Thalheimer and Rothschild<sup>90</sup> studied heart lesions in rabbits inoculated with *S. viridans*, with Poynton and Paine's strains of *S. rheumaticus*, and with strains of the epidemic sore-throat streptococci (the latter two had been cultivated some time). They described various types of myocarditis, among others areas of small-round-cell infiltration, as described by Bracht and Wächter, and also proliferative lesions, in which there was primarily a degeneration of muscle fibers, "but fibroblasts soon become prominent and develop rapidly to a fibrous stage." These latter they believed did not resemble Aschoff-Geipel bodies because "they show no predilection for a subendocardial situation nor for the bases of the cardiac valves," because giant cells were absent, and because the cells of which they were composed did not stain so deep a red with pyronin as do the Aschoff cells. Hartzell and Henrici<sup>90</sup> described myocardial changes of various kinds occurring in rabbits inoculated with strains of *S. viridans* from dental abscesses and pyorrhea alveolaris. In 2 of these rabbits there developed lesions of a proliferative character, in which multinucleated giant cells were prominent, and which they believed were similar to Aschoff-Geipel bodies.

<sup>58</sup> Jour. Med. Research, 1915, 31, p. 455.

<sup>59</sup> Jour. Exper. Med., 1914, 19, p. 429.

<sup>60</sup> Jour. Am. Med. Assn., 1915, 64, p. 1055.

TABLE 5  
STREPTOCOCCUS SALIVARIUS

| Strain | Source                                 | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days | Died or Chloroformed* |
|--------|--|-----------------|--------|--------------|-----------------------------|-----------------------|
| 5      | Cervical lymph node.....               | 3               | 26     | 3            | 5                           | 0                     |
|        |  |                 | 27     | 5            | 5                           | +                     |
|        |  |                 | 28     | 15           | 3                           | 0                     |
|        |  |                 | 29     | 45           | 1                           | +                     |
| 9      | Tonsil.....                            | 7               | 41     | 5            | 1                           | 0                     |
|        |  |                 | 42     | 5            | 2                           | 0                     |
|        |  |                 | 43     | 5            | 3                           | 0                     |
|        |  |                 | 44     | 5            | 3                           | 0                     |
|        |  |                 | 45     | 5            | 3                           | 0                     |
|        |  |                 | 46     | 5            | 3                           | 0                     |
| 19     | Pyorrhea alveolaris.....               | 4               | 53     | 5            | 4                           | 0                     |
|        |  |                 | 54     | 5            | 4                           | 0                     |
|        |  |                 | 55     | 5            | 4                           | 0                     |
|        |  |                 | 56     | 5            | 4                           | 0                     |
| 25     | Dental abscess.....                    | 8               | 78     | 5            | 6                           | 0                     |
|        |  |                 | 79     | 5            | 6                           | 0                     |
|        |  |                 | 80     | 5            | 6                           | 0                     |
|        |  |                 | 81     | 5            | 6                           | 0                     |
| 35     | Strain 19 after one animal passage     | 3               | 115    | 45           | 2                           | +                     |
|        |  |                 | 116    | 5            | 2                           | 0                     |
|        |  |                 | 117    | 5            | 1                           | +                     |
|        |  |                 | 118    | 5            | 2                           | +                     |
| 56     | Feces.....                             | 11              | 171    | 5            | 8                           | 0                     |
|        |  |                 | 172    | 8            | 8                           | 0                     |
|        |  |                 | 173    | 5            | 8                           | 0                     |
|        |  |                 | 174    | 10           | 9                           | 0                     |
| 64     | Blood culture (malignant endocarditis) | 7               | 175    | 5            | 8                           | 0                     |
|        |  |                 | 177    | 10           | 8                           | 0                     |
|        |  |                 | 178    | 5            | 8                           | 0                     |
|        |  |                 | 179    | 8            | 8                           | 0                     |
| 95     | Dental abscess.....                    | 1               | 202    | 3            | 3                           | 0                     |
|        |  |                 | 203    | 5            | 1                           | +                     |
|        |  |                 | 204    | 8            | 1                           | +                     |
| 24     | Tonsil.....                            | 8               | 73     | 75           | 2                           | 0                     |
|        |  |                 | 74     | 30           | 6                           | 0                     |
|        |  |                 | 75     | 5            | 6                           | 0                     |
|        |  |                 | 76     | 5            | 6                           | 0                     |
|        |  |                 | 77     | 5            | 2                           | 0                     |

\* See Table 1 for the significance of + and H.

The heart lesions which occurred frequently in the rabbits of this study varied widely in character, presenting but one feature in common, namely, their focal nature. There were small areas of necrosis in the muscle fibers, frequently containing masses of cocci, and at times unaccompanied by other changes save for interstitial hemorrhages; then there occurred, in addition to this condition, varying degrees of polymorphonuclear infiltration, up to the development of well-marked miliary abscesses. These lesions we may classify in one group as degenerative-exudative.

TABLE 5—Continued  
STREPTOCOCCUS SALIVARIUS

| Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys | Remarks                                    |
|--------------|--------------|--------|-------------------|-------------|--------|---------|--|
| 0            | 0            | +      | 0                 | 0           | 0      | 0       | Osteomyelitis of tibia<br>Bronchopneumonia |
| +            | 0            | 0      | 0                 | +           | +      | 0       |  |
| 0            | 0            | 0      | 0                 | +           | +      | +       |  |
| 0            | 0            | 0      | H                 | +           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | +      | 0                 | 0           | 0      | +       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| +            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       | Subacute meningitis ("chorea")             |
| 0            | 0            | 0      | H                 | 0           | 0      | 0       |  |
| +            | +            | +      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |  |
| +            | +            | +      | 0                 | +           | 0      | +       |  |
| 0            | 0            | 0      | 0                 | +           | +      | 0       |  |
| 0            | +            | +      | 0                 | 0           | 0      | +       | Subacute meningitis ("chorea")             |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | +      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | +       | Subacute meningitis ("chorea")             |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | H            | +      | H                 | 0           | 0      | 0       | Subacute meningitis ("chorea")             |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | H      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |
| 0            | 0            | 0      | 0                 | 0           | 0      | 0       |  |

A second group consists of lesions in which lymphocytes, with occasionally plasma cells, were the principal or only infiltrating cells. Here there was little evidence of degeneration. The cells collected at times about the vessels, more frequently in spaces between the muscle fibers. They occurred in all parts of the heart.

The third group is composed of lesions that were essentially proliferative in character. Here, as in the case of the first type, the lesions varied widely. What we consider to be the acute stage of this condition consisted of an area

TABLE 6  
STREPTOCOCCUS FÆCALIS

| Strain | Source                                      | Days Cultivated | Rabbit | Dose in c.c. | Duration of Infection, Days |
|--------|---|-----------------|--------|--------------|-----------------------------|
| 3      | Saliva.....                                 | 3               | 18     | 1            | 2                           |
|        |   |                 | 19     | 5            | 2                           |
|        |   |                 | 20     | 15           | 2                           |
|        |   |                 | 21     | 45           | 2                           |
| 40     | Feces.....                                  | 3               | 128    | 30           | 7                           |
|        |   |                 | 129    | 10           | 7                           |
|        |   |                 | 130    | 8            | 7                           |
|        |   |                 | 131    | 5            | 7                           |
| 42     | Feces.....                                  | 3               | 136    | 30           | 7                           |
|        |   |                 | 137    | 10           | 7                           |
|        |   |                 | 138    | 8            | 7                           |
|        |   |                 | 139    | 5            | 7                           |
| 62     | Dental abscess.....                         | 6               | 162    | 9            | 3                           |
|        |   |                 | 163    | 6            | 3                           |
|        |   |                 | 164    | 5            | 3                           |
|        |   |                 | 165    | 5            | 3                           |
| 70     | Blood culture (malignant endocarditis)..... | 3               | 180    | 10           | 1                           |
|        |   |                 | 181    | 10           | 1                           |
|        |   |                 | 182    | 10           | 1                           |
|        |   |                 | 183    | 10           | 1                           |
|        |   |                 | 184    | 10           | 1                           |

\* See Table 1 for the significance of + and H.

of necrosis in the muscle fibers. Surrounding the dead muscle cells was an aggregation of large round or more commonly oval and spindle-shaped cells, containing large clear nuclei and having an abundant protoplasm, which tended to take the basic stains. These cells were very probably of fibroblastic origin; in fact, in some of the lesions there were a number of cells which looked like fibroblasts that showed mitotic figures. These cells stained red with pyronin-methyl green (the tissues were all fixed in Helley's fluid). Where necrosis was present there were also usually a few polymorphonuclear leukocytes, and in a few instances we found small abscesses in the heart muscle surrounded by a wide zone of cells such as have just been described. More commonly, however, there was no necrosis, and the large cells were collected between the muscle fibers, spreading them apart and thus forming fusiform masses. They also in places collected about vessels, the cells apparently being derived from the adventitia of the latter, and here the lesions could not be differentiated from those typical of rheumatic fever as described by Aschoff. These proliferative lesions were noted frequently beneath the endocardium and pericardium, and especially near the bases of the valves. In the latter situation multinucleated giant cells were particularly frequent, altho they were noted in other areas also. Many of the subendocardial lesions contained masses of hyaline material, probably altered fibrin.

In short, we repeatedly produced in rabbits, by the inoculation of streptococci, lesions which could not be histologically differentiated from the type of myocarditis generally accepted as specific for rheu-

TABLE 6—*Continued*  
STREPTOCOCCUS FÆCALIS

| Died or Chloroformed* | Heart Muscle | Heart Valves | Joints | Voluntary Muscles | Lymph Nodes | Spleen | Kidneys |
|-----------------------|--------------|--------------|--------|-------------------|-------------|--------|---------|
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | H      | H                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | H                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | +      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | +      | 0                 | 0           | 0      | 0       |
| 0                     | +            | 0            | +      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | +      | 0                 | 0           | 0      | 0       |
| 0                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| 0                     | +            | 0            | +      | +                 | 0           | 0      | +       |
| 0                     | +            | 0            | +      | 0                 | 0           | 0      | 0       |
| 0                     | +            | +            | 0      | 0                 | 0           | 0      | +       |
| 0                     | +            | 0            | 0      | 0                 | 0           | 0      | +       |
| +                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| +                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| +                     | 0            | 0            | 0      | 0                 | 0           | 0      | 0       |
| +                     | +            | 0            | 0      | H                 | 0           | 0      | 0       |
| +                     | 0            | 0            | 0      | H                 | +           | 0      | 0       |

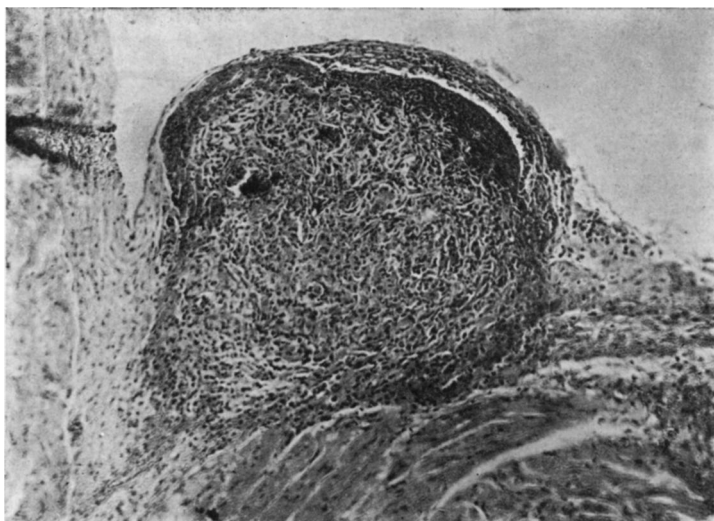


Fig. 4. Subendocardial focus of proliferative myocarditis, showing fibrin.

matic fever. Some of the lesions were atypical in the occurrence of considerable necrosis and the admixture of other types of inflammatory cells; perhaps these cells did not stain so deep a red with pyronin as do those in the human heart. We hold that these differences are not so great but that they can be explained by differences in the degree of infection between the inoculated rabbit and the man infected with the rheumatic virus, or difference in the constitution of the cells as they occur in rabbits and in man. In the general appearance of the cells, their basicity, their accumulation in fusiform masses between the fibers or in rosettes about the vessels, their frequent subendocardial position, and the frequent occurrence of multinucleated giant cells, these lesions presented all the characteristics of the typical Aschoff-Geipel nodules.

In Table 7 are indicated the percentages of rabbits which developed these different types of myocarditis after inoculation with the various strains of streptococci.

TABLE 7

THE PERCENTAGES OF RABBITS WHICH DEVELOPED DIFFERENT TYPES OF MYOCARDITIS AFTER INOCULATION WITH VARIOUS STRAINS OF STREPTOCOCCI

|                                    | Exudative<br>% | Lympho-<br>cytic<br>% | Prolifer-<br>ative<br>% | Total<br>% |
|------------------------------------|----------------|-----------------------|-------------------------|------------|
| <i>S. pyogenes</i> , Group I.....  | 5              | 8                     | 2                       | 15         |
| <i>S. pyogenes</i> , Group II..... | 15             | 10                    | 15                      | 40         |
| <i>S. anginosus</i> .....          | 8              | 7                     | 4                       | 19         |
| <i>S. mitis</i> .....              | 6              | 10                    | 15                      | 31         |
| <i>S. salivarius</i> .....         | 5              | 8                     | 0                       | 13         |
| <i>S. faecalis</i> .....           | 5              | 9                     | 14                      | 28         |
| Hemolytic strains.....             | 9              | 9                     | 7                       | 25         |
| Nonhemolyte strains.....           | 5              | 9                     | 10                      | 24         |

It will be seen from Table 7, that while variations occur, they are not sufficiently marked to establish specificity. Especially when the hemolytic strains are compared with the viridans group, do the differences become slight; and it is noteworthy that the totals indicate that neither group has a greater affinity for the heart muscle than the other.

*Aorta*.—Klotz<sup>61</sup> described fatty changes and connective-tissue increase in the intima of the arch of the aorta of rabbits repeatedly inoculated with streptococci of low virulence. In later papers<sup>62</sup> he described lesions of the arch of the aorta which are constantly present in rheumatic fever. These consisted of small foci of lymphocytic and

<sup>61</sup> Brit. Med. Jour., 1906, 2, p. 1767.

<sup>62</sup> Tr. Assn. Am. Phys., 1912, 27, p. 181. Jour. Path. and Bacteriol., 1913, 18, p. 259.



plasma-cell infiltration about the vessels of the adventitia and in the outer part of the media; in one case an acute aneurysm developed. These findings led me to examine sections of the arch of the aorta in my rabbits.

Inflammatory changes were found in the aorta in 4 animals, all of which had been inoculated with strains of *S. mitis*. In 2 there was a general infiltration of the adventitia with large cells such as those observed in the heart muscle; within this area of proliferative change miliary abscesses occurred. The media was not involved, nor did the reaction bear any relation to the vasa vasorum. The lesions began at the aortic valve and did not extend beyond the ascending limb. In one of the rabbits there was also a small area of similar infiltration beneath the intima, just above the valve cusp. A third rabbit had a small collection of lymphoid cells in the adventitia.

Hartzell and Henrici<sup>60</sup> described lesions in the inner part of the media of 3 rabbits inoculated with streptococci from dental abscesses. These lesions consisted of an area of necrosis, with broken and twisted elastic fibrils, containing a few lymphocytes. The fact that the lesions were found in young animals and were not found in a series of controls led them to suggest that possibly they were due to the inoculated streptococci.

The vascular lesion in the fourth rabbit I consider to be an earlier stage of this process. There was here a similar destruction of tissue in the media, just beneath the intima, but the area contained a number of large mononuclear cells with some multinucleated giant cells. These cells also stained red with pyronin. A section was submitted to Dr. Klotz, who suggested that these cells were phagocytes called out by the necrotic elastic tissue fibrils. The close resemblance of this lesion to the Aschoff-Geipel nodules which were produced in the heart muscle by this same strain leads me, however, to believe that here I had a localization of the streptococci in the media.

*Joints.*—Arthritis has been produced in rabbits by Poynton and Paine,<sup>21</sup> Beattie,<sup>31</sup> Shaw,<sup>51</sup> Meyer,<sup>63</sup> Wassermann,<sup>34</sup> and Rosenow<sup>64</sup> with the streptococcus from cases of rheumatic fever, and has been considered by these observers to be evidence of the specific etiologic relation of this organism to that disease.

Cole,<sup>36</sup> however, pointed out that arthritis might frequently be produced by other streptococci; but Beattie<sup>65</sup> considered that the experimental arthritis produced by *S. rheumaticus* is different from that caused by *S. pyogenes* in that it is usually nonpurulent; he also found that this experimental arthritis resembled human rheumatism in that the condition tended to pass quickly from joint to joint and was aggravated by exposure to cold.<sup>31</sup> Poynton and Paine cultivated their "diplococcus" from cases of chronic deforming arthritis, and were able to produce a chronic form of osteo-arthritis in rabbits by inoculation.

Davis<sup>66</sup> observed that arthritis developed somewhat regularly in rabbits injected with hemolytic streptococci, but was seldom produced by strains of

<sup>63</sup> Deutsch. med. Wehnschr., 1901, 27, p. 81.

<sup>64</sup> Jour. Infect. Dis., 1914, 14, p. 61.

<sup>65</sup> Jour. Exper. Med., 1907, 9, p. 186.

<sup>66</sup> Jour. Am. Med. Assn., 1912, 58, p. 1852.

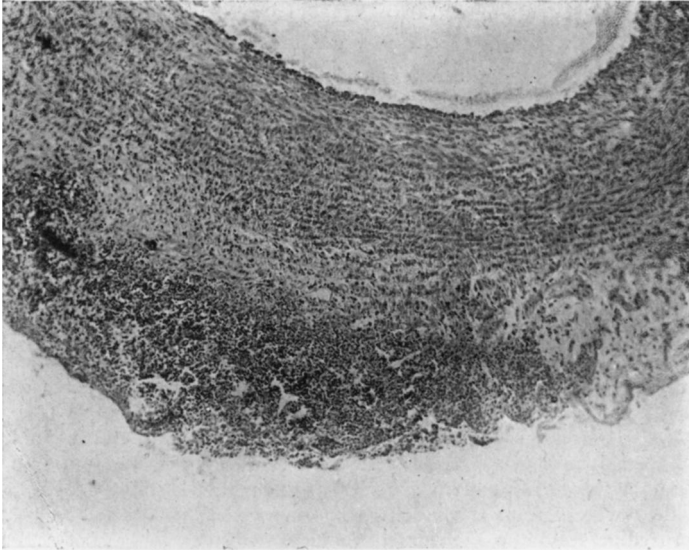


Fig. 5. Lesion in adventitia of aorta.

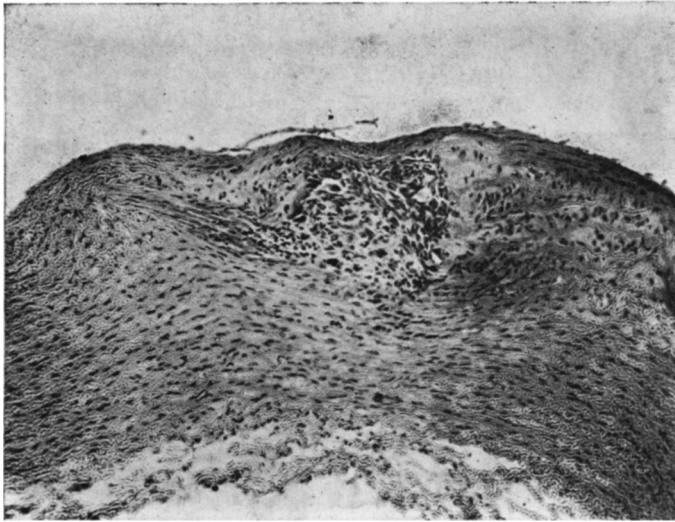


Fig. 6. Focus of proliferative inflammation in media of aorta.

*S. viridans*. Thalheimer and Rothschild,<sup>67</sup> however, found that arthritis was caused in half of their rabbits by *S. viridans*.

Coombs<sup>55</sup> noted the occurrence of "submiliary nodules," similar to those occurring in the heart muscle, in the articular tissues of cases of rheumatic fever; and Coombs, Miller, and Kettle<sup>68</sup> produced similar lesions in rabbits by inoculation with *S. rheumaticus*. Jackson<sup>69</sup> made studies of the histologic changes in the joints of rabbits produced by various streptococci, and observed similar proliferative changes. She states that "the differences in the inflammatory process in the joints of 17 rabbits of this series studied at periods varying from two hours to four months are only such as are consistent with the varying phases of a single inflammatory process, nor are there any striking differences in the reaction produced by the various kinds of streptococci employed."

Arthritis was the lesion most frequently observed in our series. In some cases there was pronounced congestion of the capsule, with hemorrhages where exudate was not present in the joint cavity; hemorrhages were especially frequent beneath the periosteum of the head of the tibia. Where arthritis was diagnosed, there was an exudate within the joint. This usually consisted of a thick mucous fluid which was turbid. Microscopical examination showed that the cloudiness was due to polymorphonuclear leukocytes. Then there were observed all degrees of severity in this condition. As the exudate increased in amount, distending the joints, it became cloudier and took on the appearance of ordinary pus. Where still more extensive involvement occurred, there was apparently considerable fibrin in the exudate, so that the joint cavities became filled with semisolid cheesy-looking material. Arthritis developed most frequently in the knee joints, next most frequently in the elbows, and occasionally in the shoulders and small joints of the paws (the latter were not examined routinely; for that reason our figures may be somewhat low). Frequently more than one joint was involved. Extensive histologic studies were not made, but sections were taken from some of the more pronounced lesions. These showed an exudation of polymorphonuclear cells in the synovial tissues, with a similar exudate on the joint surfaces. In one instance there were found multiple minute abscesses in the marrow of the tibia.

There were no pronounced differences between the percentages of arthritis produced by the various classes of streptococci, nor were there observed any differences in the character of the exudate. Joint lesions were produced with equal frequency by both hemolytic and nonhemolytic strains.

*Voluntary Muscles.*—Rosenow<sup>64</sup> called attention to lesions in the voluntary muscles produced by the inoculation of streptococci, and described similar lesions in man in cases of "muscular rheumatism" from which streptococci were isolated.

The muscle lesions in my rabbits were most frequent in the extremities, but in some instances they involved all the muscles, including the diaphragm.

<sup>67</sup> Jour. Exper. Med., 1914, 19, p. 444.

<sup>68</sup> Lancet, 1912, 2, p. 1209.

<sup>69</sup> Jour. Infect. Dis., 1913, 12, p. 364.

There were 2 types of lesions. The first group was composed of interstitial hemorrhages, and these were the more frequent. They usually occurred in circumscribed areas, but in two instances were very extensive, involving all the muscles of one extremity. The second group of lesions was rather peculiar. Grossly these appeared as small short white streaks running parallel with the muscle fibers, best seen when the rabbit was freshly killed and while the muscles still retained their translucent characters. Microscopically, these areas showed scattered isolated necrotic muscle fibers surrounded by a narrow zone of large somewhat flattened cells. There was usually no leukocytic infiltration, and this would seem to indicate that the condition was due to circulating toxins rather than to a localization of the streptococci. In a more pronounced stage of the condition a number of neighboring fibers were involved, and here the cellular reaction was more marked. The fibers were surrounded by

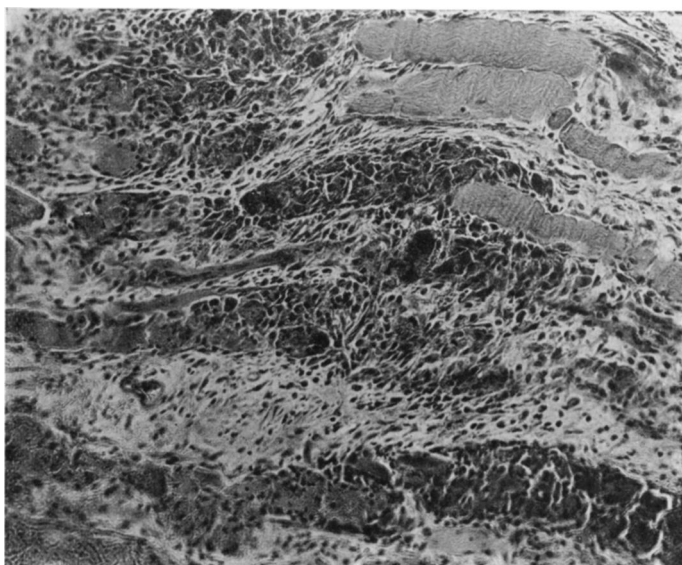


Fig. 7. Lesion in voluntary muscle, showing the breaking up of necrotic fibers and proliferative inflammation.

numbers of large oval or spindle-shaped cells. The histologic interpretation is complicated by the peculiar changes which followed the degeneration of muscle fibers, which have been described by Dawson.<sup>70</sup> Thus it becomes difficult to say which of the cells were derived from the muscles and which were of fibroblastic origin or were wandering endothelial cells. Many of the cells were in mitosis. The presence of muscle giant cells was an added striking feature. Some of the necrotic muscle fibers appeared granular and stained deeply with hematoxylin, suggesting an early deposition of lime salts. In still more marked lesions the changes described were accompanied by a leukocytic infiltration leading to the development of multiple minute abscesses.

<sup>70</sup> Jour. Path. and Bacteriol., 1909, 13, p. 174.

*Lymphadenoid Tissues.*—A number of the rabbits at autopsy showed one or more of the lymph nodes to be swollen and deeply congested or hemorrhagic. The popliteal nodes were most frequently involved, and next in frequency were the axillary glands. This is probably due to the frequency of arthritis in the knees and elbows, altho such swollen glands have been observed in the absence of any grossly visible arthritis. In some instances practically all the nodes, including the mesenteric, were involved.

Microscopically there was noted intense congestion, usually with marked hemorrhage, and pronounced changes in the parenchyma—degeneration of lymphocytes with karyolysis, a migration of cells from the adenoid tissue into the sinuses, and a disappearance of the follicles. In addition there was a pronounced increase of the endothelial cells, large numbers of which lay free in the sinuses and were ingesting nuclear fragments.

The spleen showed somewhat similar changes, save that intense congestion, hemolysis, and the deposition of fibrin were striking features; but there were noted the same tendency for the follicles to disappear, and the same increase of phagocytic endothelial cells. The condition was identical with the acute splenitis which accompanies septic processes in man. In several instances there was noted a deposition of hyaline material in the germinal centers. There were also observed several spleens containing colonies of cocci in many areas. One rabbit with endocarditis showed septic infarcts in the spleen.

In 2 rabbits inoculated with the same strain there was marked congestion with multiple punctate hemorrhages in the thymus. These rabbits had also acute splenitis and lymphadenitis. No parenchymatous changes were noted in the thymus, however.

*Kidneys.*—Various types of kidney lesions have been described by several authors studying experimental streptococcal infections in rabbits. Le Count and Jackson<sup>71</sup> made a very thorough study of these changes, and as the lesions which I found were identical in every respect with theirs, a very brief description will suffice.

While there occurred at times minute polymorphonuclear abscesses with or without the presence of bacterial emboli, the great majority of the lesions consisted of areas of lymphocytic infiltration. These were most frequently present in the cortex, and almost invariably occupied a perivascular position, either as a small nodule surrounding one of the smaller vessels, or as long radiating streaks running through the cortex at right angles to its surface. In a number of instances there was noted infiltration about the glomeruli, at times of lymphocytes, but also frequently of larger mononuclear cells tending to assume the spindle shape of the cells found in the heart and voluntary-muscle lesions. Such cells were arranged concentrically about the glomerulus.

I realize that kidney lesions of this character may occur spontaneously in rabbits, and I attempted to throw these out of my figures by not accepting any lesions where fibrosis or dilatation of tubules had occurred, since it is doubtful if such changes could develop in the streptococcal lesions in the short time these rabbits were allowed to live.

<sup>71</sup> Jour. Infect. Dis., 1914, 15, p. 389.



Fig. 8. Kidney lesion, showing a streak of lymphocytic infiltration extending across the cortex.

Kidney lesions were produced somewhat more frequently by the hemolytic strains than by *S. viridans*, but there was not observed any greater tendency of the former group to produce miliary abscesses.

*Other Tissues.*—A great variety of inflammatory changes was observed in the liver, but my rabbits were so constantly infected with coccidia that I did not feel safe in attributing any of these lesions to the streptococci. No changes were seen in the gallbladder save the results of coccidiosis. In several instances I noted punctate hemorrhages in the wall of the stomach, in one case associated with a small ulceration; microscopically, this showed free blood in the submucosa and a loss of substance in the overlying mucous membrane, with no inflammatory reaction. Two animals developed punctate hemorrhages in the submucosa of the appendix, and in one there were similar lesions in the small intestine. I repeatedly noted in sections of the appendix areas of necrosis in the follicles of the submucosa, such as have been described by Poynton and Paine<sup>71</sup> and by Rosenow,<sup>72</sup> but these always were associated with the presence of coccidia. One rabbit developed bronchopneumonia, and another showed miliary abscesses throughout the lungs.

#### ANALYSIS AND DISCUSSION OF RESULTS

Charts 1 to 3 also present the results of the experiments. These charts require a word of explanation. The column labeled "Infected" indicates the total percentage of animals which showed some evidence

<sup>72</sup> Jour. Infect. Dis., 1915, 16, p. 240.

that the inoculation resulted in infection, either in death or in the occurrence of localized lesions or both. The column marked "Died" shows the number of rabbits that died spontaneously within the time limits of the experiments. The "Septicemia" column denotes the percentage of rabbits that died spontaneously without developing lesions, usually within 24 hours; here septicemia was undoubtedly the cause of death. It is not to be inferred that these were the only rabbits that developed septicemia, as probably many of the animals which had localized lesions also had a blood infection; certainly this is true of the animals that developed splenitis.

It will be seen from Chart 1 that practically all of the various lesions were produced by one or another strain of each of the types of streptococci in the classification used. The only variations, then, were quantitative ones, and such variations were considerable. However, in order to establish the value of such quantitative differences between the various strains of streptococci in point of tissue affinity and virulence, conclusions should be drawn from a considerably larger series of experiments than is here reported. That is, while the whole number of strains and of rabbits studied was fairly large, these numbers become small in the individual groups, and, moreover, there was a disparity between the numbers in some groups and those in others. Thus Group I of *S. pyogenes* was represented by 11 strains that were inoculated into 46 rabbits; Group II, by 5 strains and 20 rabbits; *S. anginosus*, by 6 strains and 26 rabbits; *S. mitis* by 17 strains and 74 rabbits; *S. salivarius*, by 9 strains and 38 rabbits; and *S. faecalis*, by 5 strains and 21 rabbits.

The quantitative variations, probably for the reasons just given, having occurred in a sort of "hit or miss" manner, do not lend themselves readily to analysis, especially since each of the groups has been classified by the use of 2 characteristics, their hemolytic powers and their ability to ferment carbohydrates. We may study the influence of each of these factors separately by combining those strains fermenting the same carbohydrates, irrespective of hemolysis, and vice versa.

In Chart 2 is shown the relation of the power of sugar-fermentation to virulence and elective organ affinities. The 1st group, consisting of strains fermenting both salicin and mannite, contains *S. pyogenes*, Group II, and *S. faecalis* of the former classification; this group represents 10 strains and 41 rabbits. The 2nd group is composed of strains fermenting salicin alone, and contains *S. pyogenes*, Group I, and *S. mitis*. This group contains the majority of strepto-

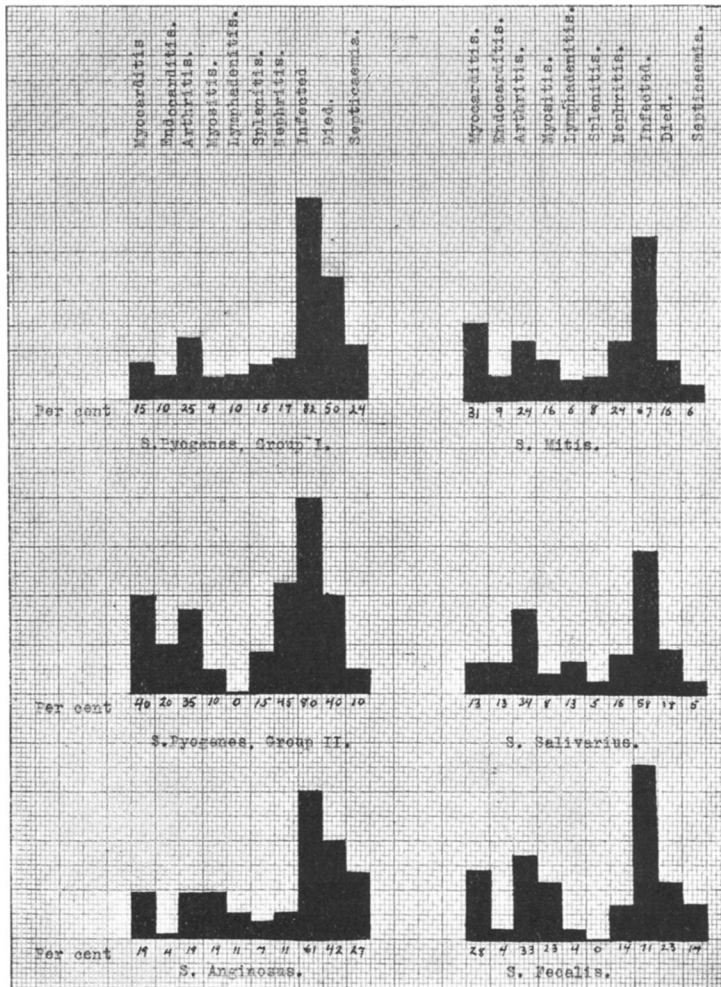


Chart 1. A comparison of the total percentages of rabbits inoculated as indicated, that showed (1) lesions in various organs, (2) evidence that the inoculation resulted in infection (either by death or the occurrence of localized lesions or both), (3) that died spontaneously within the limits of the experiment, and (4) that died of septicemia, without developing lesions.

cocci pathogenic for man. There were 28 strains of the second group inoculated into 120 rabbits. The last group, fermenting neither of the two carbohydrates, contains *S. anginosus* and *S. salivarius*; this group is composed of 15 strains which were injected into 64 rabbits.

In this chart the number of observations in each group is larger and it will be seen that pronounced variations do not occur. Espe-



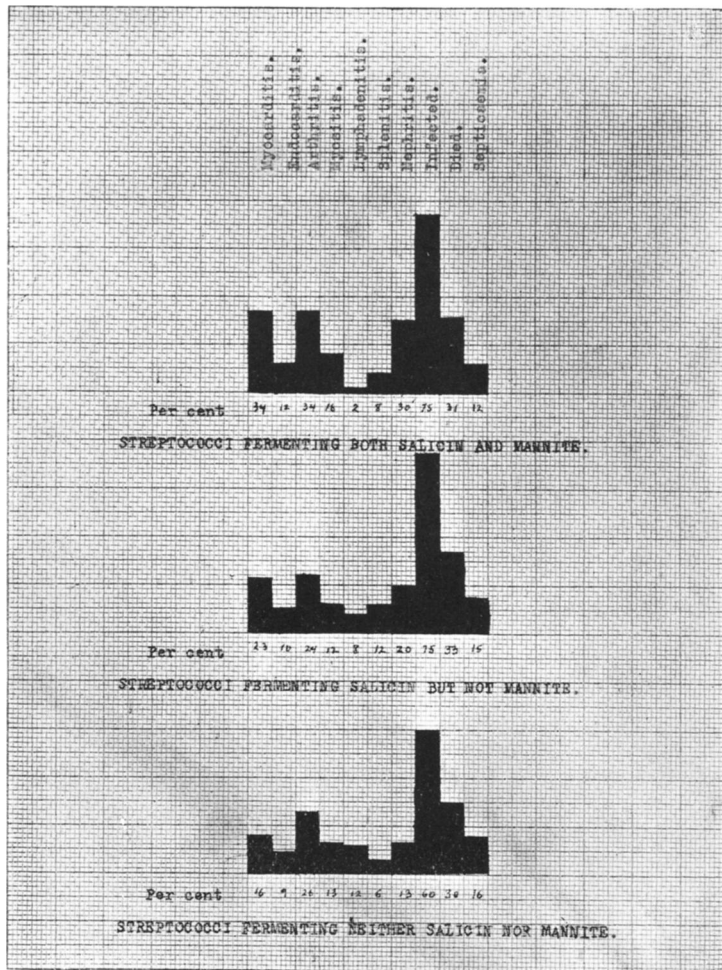


Chart 2. The relation of the sugar-fermenting power to virulence and elective organ affinities.

cially the virulence, as measured by the percentages of spontaneous deaths and septicemias, remains practically constant. There is a slight tendency to a decrease in all the localized infections (especially in the myocardial and renal lesions) as we pass from the 1st to the 3rd groups. This variation, however, is not pronounced and its significance is doubtful.

Chart 3 presents the results obtained when the hemolytic strains are compared with the nonhemolytic irrespective of the sugar tests.

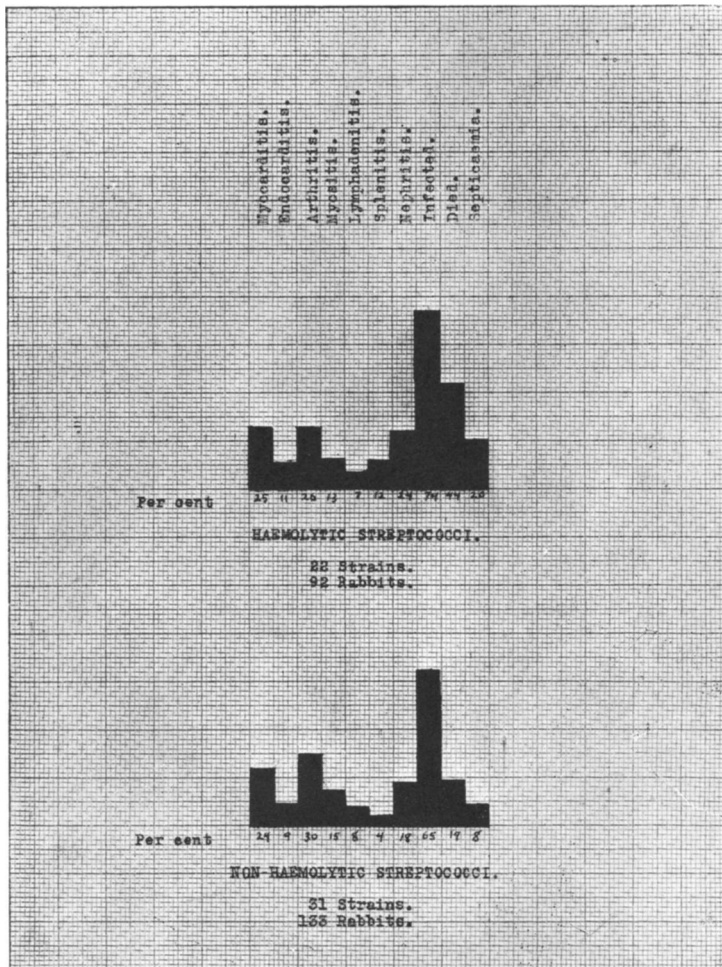


Chart 3. The relation of the hemolytic power of streptococci or the lack of it to virulence and elective organ affinities.

This chart shows two striking features. The hemolytic strains are far more virulent, as shown by the much higher percentages of spontaneous deaths and of septicemias, and also by the much greater incidence of acute splenitis. The other figures, denoting the occurrence of localized lesions, remain practically the same in the two groups.

The figures in the first four columns, denoting the incidence of the so-called rheumatic lesions, are particularly significant, for they indi-

cate that these lesions are produced with practically identical frequency by both *S. hemolyticus* and *S. viridans*. This is not in accord with what we have been led to believe from previous literature, nor with clinical experience. For certainly we find nonhemolytic strains associated with endocarditis, at least, far more frequently than we do hemolytic ones. It is suggested that the lesions produced in rabbits may be no indication of the tissue affinities of the same strains in man.

#### SUMMARY AND CONCLUSIONS

Fifty-three strains of streptococci from various sources were inoculated into 225 rabbits, and the virulence and elective organ affinities were compared with the powers of hemolysis and carbohydrate-fermentation.

The carbohydrate-fermentation tests are of no significance from the standpoint of virulence, and of slight or doubtful significance from the standpoint of tissue localization.

The hemolytic streptococci are more virulent than the non-hemolytic, but the two classes localize in the same tissues with equal frequency.

We are not justified, from evidence obtained by rabbit-inoculation experiments, in recognizing any particular class of streptococci as specific for rheumatic fever, since the various rheumatic lesions, arthritis, myocarditis, endocarditis, and myositis, may be produced by some strains in each of the varieties, and are produced in equal proportion by both hemolytic and nonhemolytic streptococci.

Streptococci of various kinds may produce in rabbits types of myocarditis which cannot be differentiated from the Aschoff-Geipel nodules generally considered diagnostic of rheumatism.

Experimental streptococcal endocarditis usually develops by implantation rather than by embolism.